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The Role Of Annona Squamosa In Mitotic Inhibition: A Comparative Study With Methotrexate Using Fenugreek Seeds"

Vijaya Ambica Durga Bonthu [1 *], Bhaskararaju Vatchavai [2], Jyothsna Satya Priya Konala [3] Tejasri Lakshmi Kunche [4], Pujitha Sattineedi [5], Shafiya Shaik [6], Dhanusree Seelamsetti [7].

[1 *] Associate Professor, [2] Principal , [3,4, 5, 6,7]- Student

Department of Pharmacology

Sri Vasavi Institute of Pharmaceutical Sciences, Pedatadepalli, Tadepalligudem India.

ABSTRACT:-

The present study compares the antimitotic activity of Annona squamosa leaf extract to Methotrexate utilising a fenugreek seed germination experiment. The inhibition of root elongation was employed to detect inhibited cell division. Phytochemical screening of the extract revealed the presence of biologically active substances such as alkaloids, flavonoids, tannins, and glycosides. Methotrexate and Annona squamosa extract slowed root development in a dose-dependent manner. Although methotrexate showed greater inhibition, the extract also had significant antimitotic effects, particularly at higher dosages. These findings indicate that Annona squamosa has significant potential as a natural source of antimitotic drugs.

Keywords:

Annona squamosa, Antimitotic activity, Methotrexate, seed germination assay, Trigonella foenum-graecum.

INTRODUCTION:-

Annona squamosa, often known as sugar-apple or custard apple, is a tropical fruit-bearing plant from the Annonaceae family. A. squamosa, which originated in the Americas, is now grown in many parts of the world for its delectable fruits and therapeutic benefits. The plant is distinguished by its small, round, or heart-shaped fruits with a knobby, greenish-yellow surface and creamy white flesh that contains numerous black seeds[1].

In addition to its culinary applications, A. squamosa has a long history of traditional medical use in various cultures. Traditional medicine has used many plant parts, such as the leaves, stems, bark, and seeds, to treat a variety of health challenges [2,3]. Traditional treatments developed from A. squamosa are said to address diseases such as gastrointestinal disorders, respiratory infections, inflammatory conditions, and diabetes.

Many of A. squamosa's traditional applications have been confirmed by modern scientific study, which has revealed its pharmacological qualities and mechanism of action. Extracts and bioactive chemicals extracted from various portions of the plant have demonstrated a wide range of pharmacological properties, including antioxidant, anti-inflammatory, antibacterial, antidiabetic, anticancer, and antihypertensive actions. These pharmacological qualities have drawn the interest of researchers and pharmaceutical companies, prompting deeper inquiry into the therapeutic potential of A. squamosa in the development of innovative medicines [4,5].

PLANT DESCRIPTION:

SYNONYMS:-

Annona squamosa-synonyms are annona cinerea, annona glabra, annona squamosal var.glabra, custard apple, sugar apple, sweetsop

This includes the fresh and dried whole plant of the Annonaceae family group of flowering plants commonly know as custard apple family.

Fig:1 Annona squamosa

MORPHOLOGY:-

The custard apple, also known as Annona squamosa, is a tropical fruit tree with unique physical characteristics. The tree can grow to be 10 to 15 meters tall and has a straight trunk covered in smooth, greyish-brown bark. The drooping, slender branches are covered with soft, hairy vegetation. The leaves are 10-20 cm long and 4-8 cm wide, with an elliptical shape and an alternating pattern. They have a smooth, waxy texture and are pale green on the bottom and dark green on top. Each flower is composed of three sepals and three petals [6,7].

Syncarps are round or heart-shaped fruits that form when many ovaries fuse together. They have a diameter of 5-10 cm and are green when unripe, becoming yellow or purple when full. The fruit's white, creamy, and delectable pulp contains a handful of black seeds. Annona squamosa's morphology is defined by its unusual tree shape, leaf structure, flower arrangement, and fruit features [8,9].

Table-1: Morphology

shape	Simple,alternate elliptical
Size	10-20 cm long,4-8 cm wide
colour	Dark green
odour	Pleasant
taste	Sweet

TAXONOMY:-

Kingdom : Plantae

Subkingdom : Tracheobionta

Super division : Spermatophyta

Division : Magnoliophyta

Class : Magnoliopsida

Sub class : Magnoliidae

Order : Magnoliales

Family : Annonaceae

Genus : Annona

Species : squamosa

PHYTOCONSTITUENTS:-

Phytochemicals found in A. squamosa L. leaf and seed extracts accounted for a sizable portion of the plant's chemical makeup, according to research (Al Kazman et al., 2022). According to Leite et al. (2021), these phytochemicals comprised phenolics, peptides, acetogenins, triterpenoids, alkaloids, essential oils, and fixed oils.

The main constituents of Annona squamosa:

1.Annonaine: An alkaloid with antibacterial, anti-inflammatory, and antioxidant properties.
2.Squamocin: A glycoside with cytotoxic and antitumor properties.
3. Annonin: This terpenoid is said to have anti-inflammatory and antioxidant qualities.

Table-2: chemical constituents

constituent	Type	Functions
Annonaine	Aporiphine alkaloid	Anti bacterial,anti depressant
Squamocin	Annonaceous acetogenin	Anti cancer, cell cycle arrest
Annonin	Acetogenin	e^-transport chain in mitochondria
Gallic acid	Phenolic acid	Anti oxidant

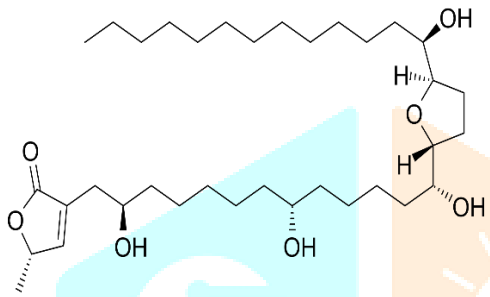
Beta carotene	Lipophilic	Dietarysource ,skinhealth, immune system
Asimicin	Actogenin	Anti tumour, pesticidal
Lycopene	Tetraterpene	Anti inflammatory ,vision

LEAVES:

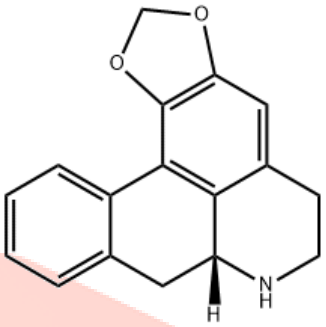
1.Alkaloids: annonaine, annomontine

2.Flavonoids: quercetin, kaempferol

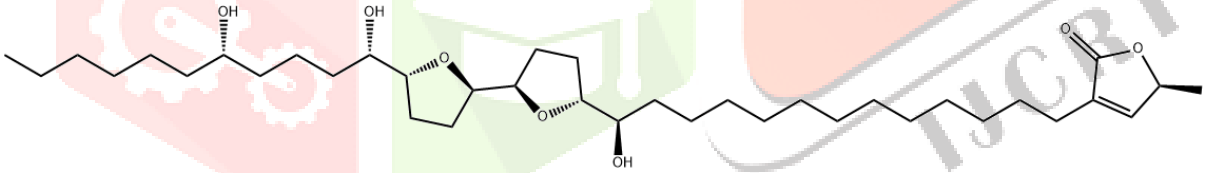
3.Phenolic acids: gallic acid, ellagic



Annonacin



Annonaine



Squamocin

MATERIALS AND METHODS:-

Plant material collection and preparation: Annona squamosa leaves were taken from a local garden and properly washed with distilled water to eliminate dirt and contaminants. The leaves were air-dried in a shaded area before being pulverised into a fine powder with a mechanical grinder.

PREPARATION OF EXTRACT:-

The powdered leaves were extracted with ethanol as a solvent. In a clean glass container, around 100 g of powdered leaves were mixed with 250 mL of ethanol [30:70]. The mixture was macerated for 72 hours at room temperature, with occasional stirrings. Following extraction, the solvent was evaporated at reduced pressure with a rotary evaporator to produce a concentrated leaf extract. The dried extract was stored in an airtight container and tested.

Fig:2 Extract preparation

PHYTOCHEMICAL SCREENING:-

Sample solution is extract solution[Annona squamosa].

Test for alkaloids:

Mayer's test: Add a few drops of Meyer's reagent (potassium mercuric chloride solution) to 1ml of each sample solution. A creamish white precipitate appeared, indicating the presence of alkaloids.

Tests for tannins:

Lead acetate test: When test samples were mixed with lead acetate solution, they formed a white precipitate.

Ferric chloride test: When a few drops of ferric chloride were added to a few ml of separately collected test samples, a blackish crimson precipitate appeared.

Test for glycosides:

Legal test: When samples are treated with pyridine and sodium nitroprusside solution, they turn blood red.

Kellar-Kiliani test: A blue colour was generated when one ml of concentrated sulphuric acid was poured into a test tube, followed by five ml of extract, two ml of glacial acetic acid, and a single drop of ferric chloride.

Test for saponins:

Foam test: 5ml of extract was vigorously shaken to form a stable, long-lasting foam. Saponins could be identified by combining the froth with three drops of olive oil and observing the formation of an emulsion.

Test for flavonoids:

Alkaline Reagent Test: Add NaOH solution; a bright yellow colour develops, which diminishes after acid addition.

Test for fixed oils:

Spot test: Place 1-2 drops of sample solution on a piece of dry filter paper and dry it at room temperature for 15-20 minutes. If a transparent, greasy patch remains after drying, it indicates the presence of fixed oils.

DETERMINATION OF ANTIMITOTIC ACTIVTY:-

ANTI MITOTIC ACTIVITY:-

The inhibition of mitosis is a critical mechanism in cancer therapy, and several natural plant-derived compounds have demonstrated promising antimitotic potential. Methotrexate, a folate antagonist, is a well-known chemotherapeutic drug that inhibits DNA synthesis, thereby arresting cell division. **Annona squamosa** (custard apple), a traditional medicinal plant, contains phytoconstituents such as acetogenins, alkaloids, and flavonoids, which may exert cytotoxic or antiproliferative effects.

The seed germination assay provides a simple and effective model to assess the antimitotic effects of compounds using meristematic root tissues. This study evaluates the comparative effects of A. squamosa leaf ethanolic extract and methotrexate on seed germination and early seedling growth in fenugreek seeds (Trigonella foenum-graecum).

Materials required:-

- *Annona squamosa* (custard apple) fresh leaves
- Methotrexate (standard drug)
- Fenugreek seeds (Trigonella foenum-graecum)
- Distilled water
- Whatman filter paper or cotton
- Petri dishes
- Normal room temperature setup
- Measuring scale

Stock preparation of Annona squamosa ethanolic extract:-

The stock solution of Annona squamosa ethanolic extract were prepared by dissolving 1g of extract in 10 ml of distilled water from which serial dilutions of 25, 50, 100 µg/ml were prepared.

Stock preparation of Standard drug:-

The stock solution of standard drug was prepared by dissolving 15mg of methotrexate in 10 ml of water from which serial dilutions of 25, 50, 100 µg/ml were prepared.

Seeds preparation:-

Seeds of Trigonella foenum-graecum used in the study were obtained from local store, dry seeds of equal weight were sterilized and followed by rinsing with sterile distilled water[4-5 times].

Assay procedure:-

The prepared dilutions were taken in petri plates and sterilized dry seeds were added in each petriplate, incubating at room temperature for 72 hr.

⇩

Seeds were soaked in tap water in the control group for 72 hr.

⇩

For morphological studies, photographs were taken. Percentage of seed germination was evaluated in control, plant extract and standard drug. Water is used for dilution.

Water as a control and methotrexate as a standard was used for study. Each experiment was performed in triplicate and mean value was computed by using the formula,

$$\%\text{Inhibition} = Lc - Lt / Lc \times 100$$

Where, Lc = length of control

Lt = length of test

RESULTS AND DISCUSSIONS:-

PHYTOCHEMICAL SCREENING:-

Table-2: results of phytochemical screening

Phytochemical Group	Result
Alkaloids	+
Flavonoids	+
Tannins	+
Saponins	+
Glycosides	+
Fixed Oils and Fats	–

Antimitotic Activity on Fenugreek Germination:-

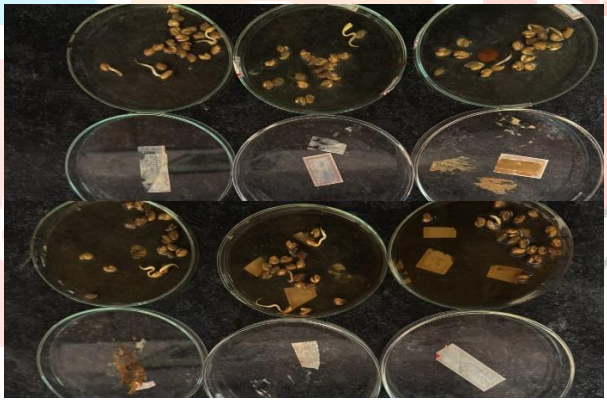
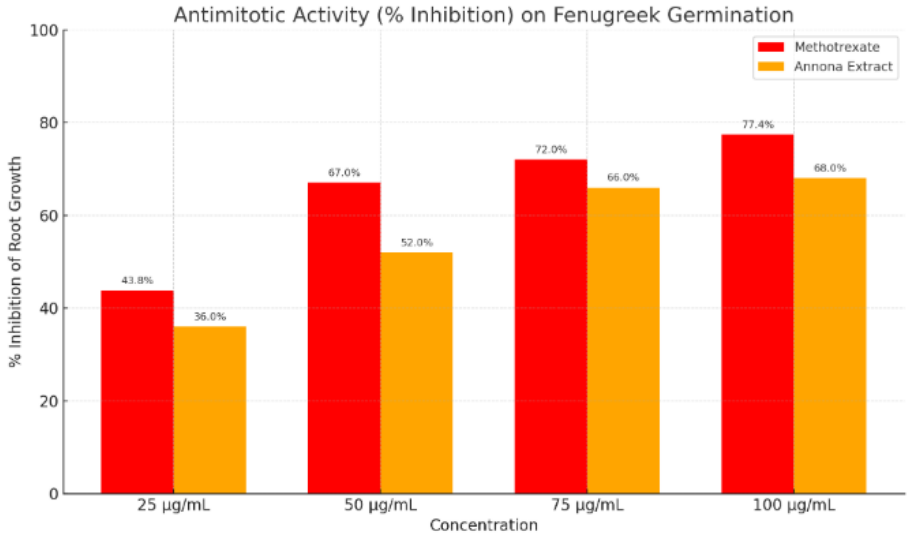


Fig:3 Results of Fenugreek seeds

Table-3: Results of Antimitotic Activity:-

Concentration (µg/mL)	Root Length (cm) – Methotrexate	% Inhibition – Methotrexate	Root Length (cm) – Annona Extract	% Inhibition – Annona Extract
25	2.81	43.8%	3.20	36.0%
50	1.65	67.0%	2.40	52.0%
75	1.40	72.0%	1.70	66.0%
100	1.13	77.4%	1.60	68.0%



DISCUSSIONS:-

The present study investigates the antimitotic potential of Methotrexate (standard) and Annona squamosa extract by evaluating their effect on fenugreek (Trigonella foenum-graecum) seed germination. The primary indicator of antimitotic activity was the inhibition of root elongation, which correlates directly with cell division at the root apical meristem.

Results demonstrated a dose-dependent inhibition of root growth by both Methotrexate and Annona extract. Methotrexate, a well-known antimitotic agent, served as the positive control and showed significant inhibition, ranging from 43.8% at 25 µg/mL to 77.4% at 100 µg/mL. Similarly, Annona squamosa extract displayed promising inhibitory effects, with inhibition increasing from 36.0% to 68.0% across the same concentration range.

Although Methotrexate consistently showed stronger inhibition, Annona extract also exhibited substantial antimitotic activity, especially at higher concentrations. The difference in % inhibition at 100 µg/mL between Methotrexate (77.4%) and Annona extract (68.0%) was just 9.4%, indicating Annona's potential as a natural alternative for antimitotic interventions.

CONCLUSION:-

This study found that Annona squamosa leaf extract had significant antimitotic activity, as indicated by its inhibition of root elongation in Trigonella foenum-graecum seed germination assays. While methotrexate inhibited more strongly across all tested concentrations, the extract's comparable activity, particularly at larger doses, suggests its potential as a natural source of antimitotic drugs. These findings stimulate further investigation into Annona squamosa's bioactive substances and modes of action, which might help in the development of plant-based mitotic inhibitors.

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